Screening for Ryanodine Receptor Type 2 Mutations in Families With Effort-Induced Polymorphic Ventricular Arrhythmias and Sudden Death

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OBJECTIVES
We sought to establish the role of genetic screening for ryanodine receptor type 2 (RyR2) gene mutations in families with effort-induced polymorphic ventricular arrhythmia (PVA), syncope and juvenile sudden death.

BACKGROUND
The RyR2 mutations have been associated with PVA, syncope and sudden death in response to physical or emotional stress.

METHODS
We studied 81 subjects (39 males and 42 females; mean age 31 ± 20 years) belonging to eight families with pathogenic RyR2 mutations. All subjects underwent screening for RyR2 mutations, electrocardiography (ECG), 24-h Holter monitoring, signal-averaged electrocardiography (SAECG), two-dimensional echocardiography and exercise stress testing. Electrophysiologic (EP) study was performed in nine patients.

RESULTS
Six different RyR2 mutations were found in eight families. Forty-three family members carried the gene mutation. Of these, 28 (65%) showed effort-induced arrhythmic symptoms or signs and one died suddenly during follow-up. Family history revealed 19 juvenile cases of sudden death during effort or emotion. In two families sharing the same mutation, no subject presented with PVA during the stress test; thus, sudden death and syncope were the only clinical manifestations. The 12-lead ECG was normal in all but two subjects, whereas five patients showed positive late potentials on the SAECG. In 17 (39.5%) of 43 subjects, the two-dimensional echocardiogram revealed localized kinetic abnormalities and mild structural alterations of the right ventricle. The EP study was not able to induce PVA.

CONCLUSIONS
The absence of symptoms and PVA on the stress test in more than one-third of carriers of RyR2 mutations, as well as the lack of PVA inducibility by the EP study, underlines the importance of genetic screening for the early diagnosis of asymptomatic carriers and prevention of sudden death.

Effort-induced polymorphic ventricular arrhythmia (PVA) consists of ventricular arrhythmias with a multifocal pattern that can progress to polymorphic ventricular tachycardia in response to vigorous exercise (1). A minor form of arrhythmogenic right ventricular dysplasia (cardiomyopathy) type 2 (ARVD2) characterized by the presence of this type of effort-induced ventricular arrhythmia was described for the first time in 1988 (2). The disease locus was mapped to chromosome 1q42-q43 (3). In the same period, a series of young patients with stress-induced PVA and a normal QT interval in the absence of structural abnormalities was also described (4). Their first symptoms occurred mostly before the age of 10 years, and these arrhythmias were labeled “catecholaminergic” because they were supposedly induced by increased adrenergic stimulation.

Subsequently, linkage to chromosome 1q42-q43 was confirmed by studying new families with either structurally normal heart or mild right ventricular (RV) changes (5,6). The arrhythmias were dangerous, precipitating syncope and juvenile sudden death in response to physical or emotional stress. After the exclusion of several candidate genes mapped to 1q42-q43 (7), three different studies demonstrated the occurrence of mutations in the human cardiac ryanodine receptor type 2 (RyR2) gene in affected patients (7–9). The finding of RyR2 mutations in ARVD2, in both catecholaminergic ventricular tachycardia and familial polymorphic ventricular tachycardia, raised the question of possible allelism of these diseases. In this investigation, we assessed the role of genetic screening for RyR2 mutations in affected families.
Abbreviations and Acronyms

ARVD2 = arrhythmogenic right ventricular dysplasia (cardiomyopathy) type 2
DNA = deoxyribonucleic acid
ECG = electrocardiogram/electrocardiographic
EP = electrophysiologic
PCR = polymerase chain reaction
PVA = polymorphic ventricular arrhythmia
RV = right ventricle/ventricular
RyR2 = ryanodine receptor type 2
SAECG = signal-averaged electrocardiography

METHODS

Study group. Eight families comprising 81 subjects (39 males and 42 females; mean age 31 ± 20 years) were identified through index case analysis. The family tree was reconstructed in each family. The families had been studied following a case of juvenile sudden death after effort- or exercise-related syncope or PVA that had occurred in a family member.

Definition of PVA. The PVAs include isolated premature ventricular complexes progressing to bigeminy, multifocal premature ventricular complexes and bursts of polymorphic ventricular tachycardia. These ventricular arrhythmias are reproducible with exercise testing and progressively worsen as the work load increases (1, 10).

Study protocol. All subjects underwent 12-lead electrocardiography (ECG), signal-averaged electrocardiography (SAECG), 24-h Holter electrocardiography, exercise stress testing and two-dimensional echocardiography with Doppler study. The presence of symptoms such as syncope and lipothymia and of a family history of syncopal episodes and/or three or more consecutive premature ventricular beats (nonsustained ventricular tachycardia).

The electrophysiologic (EP) study included evaluation of the baseline conduction interval. The protocol for pacing consisted of the triple extrastimuli technique in two regions (infundibular and apical) of the RV. In three subjects, isoproterenol infusion (2 μg/min) was also administered.

The methods used for SAECG and two-dimensional echocardiography have been previously described (11). All instrumental findings were assessed blindly before the deoxyribonucleic acid (DNA) screening results.

The bicycle ergometer test was performed with continuous recording of ECG leads II, aVF and V1 with the 25 W × 2 min protocol. In subjects with effort-induced PVA, beta-blocker therapy was utilized (200 to 400 mg acebutolol or 100 mg/day atenolol), and the stress test was repeated every 6 to 12 months to assess the efficacy of antiarrhythmic therapy. The test was repeated annually in subjects <25 years of age who had no effort-induced PVA.

Peripheral blood samples were obtained from living members of each family, with informed consent, and genomic DNA was extracted according to the salting-out procedure.

Haplotype analysis. The DNA samples were analyzed by polymerase chain reaction (PCR) using markers D1S2680, ACTN2 and D1S184. The PCR reactions were performed as follows: 100 ng of genomic DNA as a template in 12.5 μl of 1× PCR buffer (16.6 mM ammonium sulfate, 67 mM Tris hydrochloride [pH 8.3], 0.01% Tween-20, 1.5 mM magnesium chloride), containing 800 nM of each oligonucleotide, 100 μM dinucleotide triphosphate and 0.5 U of ExperTaq polymerase (ExpertTeam, Venice, Italy). Cycling conditions (denaturation at 94°C for 1 min, annealing at the working temperature for 1 min, extension at 72°C for 1 min) were repeated 30 times. After PCR, 2-μl aliquots of the reaction mixture were denatured and separated on 9% denaturing polyacrylamide gel. The gels were then silver-stained and dried.

Mutation screening. The PCR reactions were performed as described previously. All RyR2 primer sequences produced in our laboratory are available at the Web site: http://telethon.bio.unipd.it/ARVDnet/molgen_arvd2.html. Each of the 105 exon was amplified from genomic DNA, purified (PCR product pre-sequencing kit; USB, Cleveland, Ohio) and sequenced using BIG DYE dyeoxy-terminator chemistry (Perkin Elmen, Wellesley, Massachusetts) on an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, California). Chromas version 1.5 software (Queensland, Australia) and LASERGENE package computer programs (DNASTAR Inc., Madison, Wisconsin) were used to edit, assemble and translate the sequences.

Mutation screening in the control group. Once a novel RyR2 mutation was detected, in order to exclude the possibility of a nonpathogenic variant, 120 genomic DNAs (240 chromosomes) from unrelated healthy control subjects from the Venetian population were analyzed by single-strand conformation polymorphism or by PCR amplification with allele-specific primers designed for the purpose. The same samples were PCR-amplified using primers for the wild type. The PCR products were separated on 2% horizontal agarose gel in 1× tris-acetate EDTA buffer.

Statistical analysis. Statistical analysis was performed using Statistica version 5.1 (StatSoft, Tulsa, Oklahoma). Comparisons between patient groups were performed using the chi-square test or Fisher exact test for categorical variables. All continuous variables were expressed as the mean value ± SD for each measurement. All tests were two-tailed, and p < 0.05 was considered statistically significant.

RESULTS

Identification of RyR2 mutations in affected family members. Screening for RyR2 mutations in families, as shown in Figure 1, succeeded in detecting two novel missense mutations (R420W and Y2392C), in addition to the four previously detected and reported elsewhere (7).
Data are reported in Table 1. In all families, the disease was inherited as an autosomal-dominant trait. Among 81 subjects who were screened, 43 were found to carry the RyR2 mutation. Twenty-three of 43 carriers have been reported previously (2–7). All mutations occurred in evolutionarily conserved regions of the protein, in domains corresponding to the cytosolic portion of the molecule. As previously reported (7), the N2386I mutation was detected in two generations and were never detected in the control group. Unfortunately, the DNA was not available, nor could their haplotype be indirectly obtained for the study. Affected subjects belonging to an apparently unrelated family (family no. 122) inherited the mutation L433P, whereas in 10 subjects, PVA progressed to nonsustained ventricular tachycardia. In one subject, sustained ventricular tachycardia was induced (Table 2). In all but three cases, the arrhythmias originated from both the left and RVs. The PVAs were related to a heart rate increase during exercise, and each subject seemed to have a reproducible threshold of heart rate at which the PVAs appeared. The average sinus rate at the onset of arrhythmias was 127 ± 14 beats/min, and in all subjects, PVA disappeared during the first minute of recovery.

Additional clinical findings. In all 43 RyR2 mutation carriers (100%), the 12-lead ECG showed normal sinus rhythm and normal atrioventricular conduction. Mild ST-segment elevation (1 mm) in the right precordial leads was present in two subjects (5%), and the QT interval was

**Table 1. RyR2 Mutations Detected in Affected Families**

<table>
<thead>
<tr>
<th>Amino Acid Change</th>
<th>Nucleotide Change</th>
<th>Exon No.</th>
<th>Consequence</th>
<th>Protein Domain</th>
<th>Family No.</th>
<th>Estimated Penetrance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2386I</td>
<td>7157A→T</td>
<td>47</td>
<td>MM FKBP12.6 domain</td>
<td>102</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>N2386I</td>
<td>7157A→T</td>
<td>47</td>
<td>MM FKBP12.6 domain</td>
<td>123</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>R176Q</td>
<td>527G→A</td>
<td>8</td>
<td>MM Cytosolic portion</td>
<td>115</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>T2504M</td>
<td>7511C→T</td>
<td>49</td>
<td>MM FKBP12.6 domain</td>
<td>123</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>T2504M</td>
<td>7511C→T</td>
<td>49</td>
<td>MM FKBP12.6 domain</td>
<td>129</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>R420W</td>
<td>1258C→T</td>
<td>14</td>
<td>MM Cytosolic portion</td>
<td>127</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>R420W</td>
<td>1258C→T</td>
<td>14</td>
<td>MM Cytosolic portion</td>
<td>127</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>L433P</td>
<td>1298T→C</td>
<td>15</td>
<td>MM Cytosolic portion</td>
<td>122</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Y2392C</td>
<td>7175A→G</td>
<td>47</td>
<td>MM FKBP12.6 domain</td>
<td>126</td>
<td>50%</td>
<td></td>
</tr>
</tbody>
</table>

*In family no. 115, the estimated penetrance for mutation T2504M was 100%; however, it was not possible to determine the influence of the additional sequence variation R176Q on the clinical phenotype.

MM = missense mutation; RyR2 = ryanodine receptor type 2.
Figure 1. Pedigrees of eight families. Family nos. 102, 123, 115 and 122 have been already reported (7). Corresponding ryanodine receptor type 2 (RyR2) mutations identified by deoxyribonucleic acid (DNA) sequence analysis are reported on the right side. (A) The DNA sequencing of subjects from family nos. 125 and 127 revealed a C→T transition, which changes codon 420 from CGG to TGG, resulting in Arg→Trp substitution. In family no. 126, DNA sequencing of RyR2 exon 47 in all family members revealed an A→G transition, leading to Tyr2392Cys substitution. The DNA sequencing of RyR2 exon 49 in subjects belonging to family no. 129 showed a C→T transition, resulting in Thr→Met substitution.
The DNA sequencing of subjects from family nos. 102 and 123 revealed an A→T transversion, which changes codon 2386 from AAC to ATC, resulting in Asn→Ile substitution. In family no. 115, DNA sequencing of RyR2 exons 49 and 8 in all family members revealed two transitions (C→T in exon 49 and G→A in exon 8), leading to Thr2504Met and Arg176Gln substitutions. The DNA sequencing of RyR2 exon 15 in subjects belonging to family no. 122 showed a T→C transition, resulting in Leu→Pro substitution.
normal in all. On the SAECG, late potentials were recorded in five subjects (12%). The Holter ECG revealed sporadic monomorphic ventricular complexes in four subjects (9%). In 17 (39.5%) of 43 gene mutation carriers belonging to four families, the two-dimensional echocardiogram revealed kinetic alterations of the RV (Table 3).

Structural abnormalities (trabecular thickening and a highly reflecting moderator band), in the setting of a normal or slightly enlarged RV, were also found in 26 affected subjects (60%). Left ventricular volume and kinetics were normal in all subjects. Right and left ventricular angiography performed in seven affected subjects confirmed the two-dimensional echocardiographic findings. In nine subjects who underwent EP study, ventricular arrhythmias were not induced, even during isoproterenol infusion. Structural abnormalities of the RV have never been detected in family members who did not carry the gene mutation.

Follow-up. Beta-blocker therapy was administered in the 26 patients with effort-induced PVA. In addition, a cardioverter-defibrillator was implanted in a patient with a previous syncopal episode and a family history of juvenile sudden death (family no. 125). The stress test, which was repeated periodically, showed the disappearance of effort-induced PVA in 17 subjects (65%) who had beta-blocker therapy. In the remaining subjects, arrhythmias were triggered at a higher level of load; thus, the antiarrhythmic drug dosage was increased. Moreover, patients were strongly advised to avoid strenuous physical activity. During follow-up (mean 6.5 years [range 6 months to 14 years]), no patient on antiarrhythmic therapy had syncopal episodes or died suddenly.

**Family history of juvenile sudden death.** Families pedigree showed that 19 patients (12 males and 7 females; mean age 20 ± 6 years) died suddenly during effort. One of them, who was considered unaffected, died during follow-up. Subsequent genetic study of blood samples obtained during life detected the presence of the RyR2 mutation running in the family (family no. 125). None of the subjects who had sudden death was receiving anti-arrhythmic therapy. Four

### Table 2. Arhythmic Symptoms in Patients Carrying RyR2 Mutations

<table>
<thead>
<tr>
<th>Family No.</th>
<th>Examined Family Members</th>
<th>Effort Juvenile Sudden Death Subjects With RyR2 Mutations</th>
<th>Mutation Carriers With Effort NSVT</th>
<th>Mutation Carriers With Effort PPVC</th>
<th>Mutation Carriers With Effort SVT</th>
<th>Mutation Carriers With Effort VT</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>26</td>
<td>4</td>
<td>12 (93%)</td>
<td>6 (43%)</td>
<td>1 (43%)</td>
<td>10 (69%)</td>
</tr>
<tr>
<td>123</td>
<td>7</td>
<td>5</td>
<td>3 (43%)</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
<td>2 (28%)</td>
</tr>
<tr>
<td>115</td>
<td>5</td>
<td>3</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>129</td>
<td>7</td>
<td>4</td>
<td>3 (43%)</td>
<td>1 (14%)</td>
<td>1 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>6</td>
<td>3</td>
<td>2 (67%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>127</td>
<td>2</td>
<td>1</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>122</td>
<td>5</td>
<td>4</td>
<td>2 (50%)</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>126</td>
<td>4</td>
<td>3</td>
<td>2 (50%)</td>
<td>2 (50%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>19</td>
<td>28 (69%)</td>
<td>15 (53%)</td>
<td>1 (2%)</td>
<td>10 (23%)</td>
</tr>
</tbody>
</table>

Data are presented as the number (%) of subjects.

ECG = electrocardiography; RyR2 = ryanodine receptor type 2; SAECG = signal-averaged electrocardiography.

### Table 3. Twelve-Lead ECG, SAECG and Two-Dimensional Echocardiographic Findings in RyR2 Mutation Carriers

<table>
<thead>
<tr>
<th>Subjects With RYR2 Mutations</th>
<th>Abnormal 12-Lead ECG</th>
<th>Positive SAECG</th>
<th>Right Ventricular Kinetic Alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family No.</td>
<td>102</td>
<td>123</td>
<td>115</td>
</tr>
<tr>
<td>Subjects With Effort NSVT</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects With Effort PPVC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects With Effort SVT</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects With Effort VT</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>28 (69%)</td>
<td>15 (53%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Data are presented as the number (%) of subjects.

ECG = electrocardiography; RyR2 = ryanodine receptor type 2; SAECG = signal-averaged electrocardiography.
subjects (21%) had syncopal episodes, whereas in the remaining 15, sudden death appeared to be the first clinical manifestation.

**Autopsy findings.** Postmortem examination was performed in seven subjects, and the findings were a structurally normal heart in three (family nos. 123, 125 and 126), mild fatty infiltration of the RV apex in three (family nos. 125 [n = 2] and 102 [n = 1]) and a typical form of fibro-fatty arrhythmogenic RV cardiomyopathy in one (family no. 115).

**DISCUSSION**

The RyR2 mutations are known to be specifically linked to effort-induced PVA, syncope and sudden death. This study reports the genetic, clinical and instrumental findings of a group of patients with the RyR2 mutation and PVAs. In contrast to a previous report (7) that specifically dealt with the basic genetic aspects of the disease, here we examined, in detail, the clinical phenotype and demonstrated the role of genetic screening for the presymptomatic diagnosis of subjects carrying the RyR2 mutation, as well as the efficacy of antiarrhythmic therapy. Moreover, a high variability in phenotypic expression among affected subjects belonging to the same family or to unrelated families was demonstrated.

**Phenotypic expression.** Recently it has been shown that mutations in the RyR2 gene are involved in the onset of PVA during effort, either in patients with a normal heart or in those with ARVD2 (7–9). Because the incidence of juvenile sudden death is very high and beta-blocker therapy seems to be effective, the early identification of subjects carrying these mutations, with prompt treatment, is crucial.

In the clinical setting, variability of a single mutation among siblings and offspring of an affected family suggests that additional factors play a role in the phenotypic expression of RyR2 mutations. The function of RyR2 can be modulated by many substances, such as endogenous/physiologic chemicals (e.g., calcium ions, lactate, palmitoyl carnitine) and exogenous/pharmacologic modulators (e.g., caffeine, sulmazole, suramin, volatile anesthetics, immunosuppressant macrolides, heparin) (12).

The finding of RyR2 mutations in ARVD2, catecholaminergic ventricular tachycardia and familial polymorphic ventricular tachycardia raised the question of possible allelism of these diseases, as occurring in malignant hyperthermia and central core disease, in which a similar genetic defect leads to subtle phenotypic variation (7–9). In particular, the clinical picture of Finnish patients with familial polymorphic ventricular tachycardia is similar to
that of our patients with ARVD2, except for the RV abnormalities that we observed on the two-dimensional echocardiogram or at postmortem analysis in more than one-third of cases. Our data support the hypothesis that the imbalance of intracellular calcium homeostasis due to the RyR2 mutation could trigger cell death, either necrosis or apoptosis (13–19), thus leading, with time, to ARVD2 in a subgroup of patients. The unusual ECG features of polymorphic ventricular tachycardia, a finding that might argue against underlying arrhythmogenic RV cardiomyopathy, was already described in the early 1980s when Morady et al. (20), reporting on a subject with this ECG pattern, argued that some patients with arrhythmogenic RV cardiomyopathy may have ventricular arrhythmias due to catecholamine-induced automaticity rather than reentry, thus explaining why ventricular tachycardia may not have the typical left bundle branch block configuration.

**Screening for mutations of the RyR2 gene.** The cardiac ryanodine receptor plays a crucial role in excitation-contraction coupling by releasing Ca\(^{2+}\) ions from the sarcoplasmic reticulum after stimulation by calcium ions entering through the dihydropyridine receptor (14–16). All the missense mutations detected in the RyR2 gene reported herein occurred in the cytosolic portion of the molecule, in domains of the protein that are crucial for the regulation of the calcium channel (17), and resulted in substitutions of amino acids highly conserved through evolution. The RyR2 mutations resulted in clustering in two regions, whereas mutations causing malignant hyperthermia or central core disease were also clustered in the corresponding skeletal muscle ryanodine receptor gene (RyR1) (18).

In the families reported in this study, six different RyR2 mutations have been identified. None of them was found in the control group, supporting the hypothesis that these genetic variants are pathogenic. As family no. 127 includes two asymptomatic individuals in whom this mutation has been found and three sudden death cases that were not genetically examined, the presence of a rare amino acid polymorphism cannot be excluded. However, this family shares the same mutation as family no. 125, in which symptomatic carriers were present and in which a common at-risk haplotype was found, thus suggesting the existence of a relatively recent, common ancestor. Further functional studies will be necessary to determine whether this amino-acid substitution is indeed a disease-causing mutation. In contrast, the RyR2 mutation T2504M occurred in two independent families showing different at-risk haplotypes. In this case, the possibility of a common, remote ancestor might be considered, but the possibility of two independent mutations affecting the same nucleotide cannot be ruled out.

**Clinical findings.** More than one-third of carriers of the RyR2 mutation did not show effort-induced PVA, syncope or juvenile sudden death. Arrhythmias were variable, ranging from isolated premature ventricular beats to sustained ventricular tachycardia. Some subjects were fully asymptomatic, whereas others showed late-onset arrhythmias in their adulthood. In two families (family nos. 125 and 127) sharing the same mutation (R420W) and a common ancestor, syncope and sudden death were the only clinical manifestations, and the stress test was unable to induce PVA. This suggests that the clinical spectrum of RyR2 mutations should include a totally asymptomatic behavior, even after stress testing.

Because routine cardiovascular diagnostic testing mostly showed normal asymptomatic carriers, a negative test in an
apparently healthy subject cannot exclude the risk of sudden death, as demonstrated by the fatal outcome of one patient previously assumed to be unaffected.

Based on the limited number of subjects sharing the same mutation, it is still not feasible to determine the predictive value of the specific mutations. However, mutation N2386I detected in family nos. 102 and 123 (total of 16 subjects carrying the mutation) seems to be associated with a high inducibility of PVA during stress testing. The penetrance of pathogenic mutations was significantly different among families in our series. At present, it is impossible to define whether this corresponds to the actual biologic properties of the different mutations, or whether it simply depends on the very small sample size. Nevertheless, statistical analysis revealed that the different penetrance of arrhythmic symptoms or signs depends neither on age nor gender.

Juvenile sudden death occurred frequently in affected families. A postmortem report was obtained only in a few cases, and tissues specimens were unavailable for genetic study, so it was impossible to directly link sudden death to RyR2 mutations. However, the presence of such a high incidence of juvenile sudden death in families with RyR2 mutations suggests that gene mutation carriers are at risk of sudden death.

As far as the pathophysiology of PVA in patients carrying RyR2 mutations, an increased calcium leak from the sarcoplasmic reticulum, due to RyR2 channel “gain of function,” has been postulated. The presence of a hyperactive RyR2 gene would provoke the onset of delayed afterdepolarizations that could trigger ventricular arrhythmias (21,22).

Conclusions. Screening for RyR2 mutations in families with effort-induced PVA and sudden death revealed a high proportion of asymptomatic carriers. The risk stratification of sudden death in asymptomatic carriers is a challenge, because the first manifestation of the disease may be fatal. The unforeseeable risk of juvenile sudden death and the proven efficacy of beta-blocker therapy underscore the role of genetic screening for early diagnosis and preventive strategies.

Acknowledgment
The authors are deeply indebted to Paola Marcon for her help in collecting familial data.

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